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CYCLES AND RHYTHMS AND THE PROBLEM OF "IMMORTALITY" IN *PARAMECIUM*

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THE recent brilliant work of Woodruff and Erdmann has thrown a flash of light upon the old question of age and death in protozoa and upon the problem of the significance of conjugation. The long, successful cultivation of *Paramecium aurelia* by Woodruff led Jennings to say:

The work of Woodruff demonstrates that the very limited periods within which Maupas and Calkins observed degeneration has no significance for the question as to whether degeneration is an inevitable result of continued reproduction without conjugation. In other words, it annihilates all the positive evidence for such degeneration, drawn from work on the infusoria. It justifies the statement that the evidence is in favor of the power of these organisms to live indefinitely, if they are kept under healthful conditions. It shows that Weismann was correct in what he meant by speaking of the potential immortality of these organisms.¹

The same work of Woodruff led Minot to say in the course of his German lectures:

Quite conclusive as to the absence of senescence are the experiments of L. L. Woodruff, who has maintained a pedigreed race of *Paramecium* for five years without conjugation.²

Woodruff also makes the statement in different publications that the cells of his pedigreed race of *Paramecium*

¹ "Age, Death and Conjugation in the Light of Work on Lower Organisms," *Pop. Sci. Mo.*, June, 1912, p. 568.

² "Modern Problems of Biology," 1913, p. 62.

aurelia possess the potentiality to perpetuate themselves indefinitely by division under proper environmental conditions. In short, his results have given almost the only experimental evidence in support of the view, advocated by Weismann, that protozoa are potentially immortal.

The importance of this generalization and the deductions from it are self evident, and it is unfortunate that so many should have advanced it before the life history of *Paramecium aurelia* was fully known. Woodruff is to be congratulated, however, in that he, with Miss Erdmann, has now worked out stages in the life history of this organism which go far in clearing up the discrepancy between his results and those obtained by Maupas and his followers.

Woodruff has carried on his pedigreed race of *Paramecium aurelia* for more than seven years with a fairly uniform division rate, subject, however, to occasional and periodic fluctuations which he calls rhythms. These correspond roughly to what I have termed cycles which end in depression periods and, unless stimulated, by death; in rhythms, however, Woodruff maintains, there is no evidence of depression.³ Recently Woodruff finds from a careful study of material fixed during the low periods of his division rate rhythms that there takes place a complete nuclear reorganization, after which the organisms continue to live with renewed vitality as shown by the ascending division rate. This process consists in the disintegration and probable absorption in the cytoplasm of the old macronucleus, one or more divisions each of the old micronuclei, degeneration of some of the products of these divisions, and the ultimate reformation of functional macronuclei and micronuclei from others. These are, in essence, the important new facts which cytological study has revealed in the life history of *Paramecium aurelia*, and some evi-

³I would like to suggest to Professor Woodruff that he work out the death rate, the data for which he can undoubtedly obtain from his records. I venture to predict he will find that the death rate rises with the decline of the division rate and during the low sweep of the rhythms.

dence is further adduced to show that similar processes occur in *Paramecium caudatum*.

While there is little to criticize in regard to the facts as described for this remarkable process, there is room for difference of opinion in regard to the conclusions which Woodruff and Erdmann draw from them. In addition to what Hertwig has already written about this work, I would call attention particularly to their conclusions concerning endomixis, parthenogenesis, conjugating and non-conjugating lines, the life cycle, and the potential immortality of *Paramecium*.

In regard to endomixis the authors state:

Since the process results in the dissemination of the material from the old macronucleus and the so-called reduction micronuclei in the cell, it gives the opportunity for a rearrangement of the molecular constitution of the cell. This involves a more profound intermingling of nuclear and cytoplasmic substances than is possible during the typical vegetative life of the cell. *Since this intermingling occurs within a cell we term this reorganization process endomixis.* Endomixis is followed by a slight acceleration of cell phenomena and a new rhythm is initiated.⁴

Further on they add:

We would therefore put emphasis on molecular rearrangement as the result common both to endomixis and to conjugation.⁵

Nearly forty years ago Engelmann interpreted conjugation in much the same way as a process of reorganization of the cell:

. . . die Conjugation der Infusorien leitet nicht zu einer Fortpflanzung durch 'Eier,' 'Embryonalkugeln' oder irgend welche andere Keime, sondern zu einem eigenthümlichen Entwicklungsprozess der conjugirten Individuen, den man als Reorganization bezeichnen kann.⁶

In several places in the same publication Engelmann speaks of physical and chemical changes as accompanying

⁴ Woodruff and Erdmann, "A Normal Periodic Reorganization Process without Cell Fusion in *Paramecium*," *Jour. Exp. Zool.*, Vol. 17, No. 4, 1914, p. 491. The italics are in the original.

⁵ *Ibid.*, p. 491.

⁶ "Ueber Entwicklung und Fortpflanzung von Infusorien," *Morph. Jahrb.*, 1, 1876, p. 628.

conjugation. A similar interpretation was given by Calkins:

. . . it is now a well-known fact that in this process of reorganization the old macronucleus fragments and ultimately disappears in the cytoplasm. This disappearance must give rise to a great increase in the nucleo-protein content of the cell, therefore to a new chemical composition of the cell as a whole. We have recently shown that, under certain conditions, nucleo-proteins (especially the purines) have a markedly stimulating effect on the rate of cell division.⁷

Now such intermingling is no more characteristic of this process of asexual reorganization than it is of the reorganization following conjugation. In both cases, as Woodruff and Erdmann show, reorganization is effected by the physical and chemical change of the old macronucleus and portions of the old micronucleus or micronuclei. The sole difference in these processes of reorganization is not to be found in the molecular rearrangement of the cell, but, as Woodruff and Erdmann state, in the presence after conjugation of a syncaryon and the nuclei derived from it. This difference, however, does not amount to much in closely related pairs in conjugation. Several observers have shown that closely related individuals, even sister cells, of *Paramecium* may conjugate, and I have followed out through 360 generations the history of such an endogamous exconjugant from a pair which came from the same ancestral cell not more than ten days prior to conjugation. There can not be a great difference in the syncaryon resulting from such a union, over the functional micronucleus had it undergone asexual endomixis. In other words, the excellent term endomixis does not indicate phenomena peculiar to asexual reorganization in *Paramecium*, but applies equally well to the process of reorganization following conjugation. The terms asexual endomixis and sexual endomixis may serve to distinguish the process of intermingling during parthenogenesis and after conjugation, respectively.

⁷ "The Paedogamous Conjugation of *Blepharisma undulans*," *Jour. Morph.* Vol. 23, 1912, p. 685.

Woodruff and Erdmann limit the application of the term endomixis to the process of reorganization without conjugation:

We therefore have employed a new term "endomixis" for the reorganization process in *Paramecium*, in preference to parthenogenesis which Hertwig applied when he incidentally noted some isolated stages of the nuclear phenomena which we have elucidated.⁸

A new name can not alter the significance of a process or phenomenon. Parthenogenesis, in its broad sense, is the development of an individual from an egg without fertilization. In the same sense that a *Paramecium* ex-conjugant develops into a new individual, so does a *Paramecium* after this process termed endomixis. Woodruff and Erdmann say:

In parthenogenesis there is a chromatin reduction which occurs and is compensated for either in the egg itself or in some later period of the life cycle of the race.⁹

The authors are not very happy in selecting this feature as distinguishing parthenogenesis from asexual endomixis, for in most cases of recognized parthenogenesis in metazoa chromatin reduction plays no part; for example, the majority of parthenogenetic eggs give off only one polar body, thus retaining in the egg the diploid number of chromosomes; others, notably the aphids and phylloxerans, do not undergo synapsis or chromatin reduction; some others it is true, give off both polar bodies and develop with the haploid number of chromosomes as is the case in bees (males), and in artificial parthenogenesis. As to the significance of parthenogenesis neither polar body formation nor chromosome reduction furnishes the key, for in many cases the eggs are predestined to parthenogenetic development long before the polar body nuclei are formed.

In regard to the reducing divisions of the chromosomes in *Paramecium* we know very little. Evidence has been adduced to indicate that the chromosomes are divided

⁸ *Ibid.*, p. 493.

⁹ *Ibid.*, p. 492.

longitudinally in both the first and the second divisions of the maturation process. The significance of the third division is as obscure in *Paramecium* as maturation is in some metazoan hermaphrodites.

In parthenogenesis, finally, we are dealing with a biological phenomenon, not with an interpretation of parthenogenesis by Winkler or Strasburger or any other individual, and to interpret this highly significant phenomenon in *Paramecium* solely in the light of such definitions, as Woodruff and Erdmann do (p. 493), does not carry conviction, nor does it conceal the real significance of the phenomenon. Asexual endomixis in *Paramecium* is parthenogenesis and nothing else, as Hertwig originally maintained in connection with these same phenomena. Nor, except for the protozoa, is it a "new type of parthenogenesis" for, if we accept conjugation as equivalent to fertilization, its analogue is shown by the majority of parthenogenetic eggs.

In regard to conjugating and non-conjugating races of *Paramecium*, Woodruff and Erdmann state:

*Thus it is proved that both the reorganization process and conjugation are potentialities of the same race—and therefore there is no evidence for the view of Calkins ('13) that conjugating and non-conjugating races of Paramecium exist, or that "apparently some paramecia are potential germ cells, others are not."*¹⁰

This is rather a sweeping generalization to draw from one pedigreed line in which conjugating animals appeared only after six years in culture. If every *Paramecium* is a potential germ cell, why was it that no pairs of conjugating *aurelia* were found during these six years? Or, in Calkins and Gregory's observations on the first 32 cells and the pure lines arising from them, all from a single ex-conjugant, why was it that all lines from one quadrant gave epidemics of conjugation whenever the test was made during a period of six months, while all other lines from the remaining three quadrants failed to give a single pair when tested under identical conditions?

¹⁰ *Ibid.*, p. 490. Italics in the original.

Woodruff and Erdmann maintain that "under just the proper conditions" conjugation will occur; this, of course, can not be denied, but the fact that under the same conditions some lines will conjugate while others will not shows a physiological difference between them which can not be gainsaid. I have no paternal jealousy whatsoever in regard to the terms "conjugating lines" and "non-conjugating lines," and am entirely willing to accept in their place any terms which indicate the physiological difference that I wished to express. I know of no terms that express the conditions adequately. Substitute for them, if more suitable, such expressions as "always ready to conjugate" and "rarely ready to conjugate." Our observations on the 32 lines certainly justify the statement that some lines in regard to conjugation, were always ready, while others were rarely ready. Woodruff and Erdmann have paid no attention to the physiological conditions which the (perhaps unfortunate) expressions "conjugating lines" and "non-conjugating lines" were meant to express. It is true that after ten months all but four of the so-called non-conjugating lines each furnished a few pairs of conjugating individuals, just as Woodruff's line did after six years, facts which show that the terms "conjugating lines" and "non-conjugating lines" as applied to races of *Paramecium*, if used at all, should be used only in respect to relative intensity of conjugating power. In this sense Woodruff's race is a non-conjugating race. We have found, furthermore, that conjugating lines have a lower vitality as measured by the division rate, and a much higher death rate, than do non-conjugating lines, all but four of the eight lines from the conjugating quadrant dying out within three months as against four of the twenty-four lines of the non-conjugating quadrants, while at the end of twenty months only one conjugating line was alive and sixteen non-conjugating lines, a mortality of 87.5 per cent. for the former and 33.3 per cent. for the latter. In *Paramecium* it is conceivable that lines with a high conjugating

power have a less well developed power of asexual endomixis than do lines that are relatively sterile, and this, correlated with their reduced vitality, if conjugation were prevented, would account for the death of all pedigreed races prior to Woodruff's, which, as Woodruff and Erdmann now show, has a high power of asexual endomixis. We are still justified, I believe, in maintaining the statement—modified now by their description of asexual reorganization—as quoted by Woodruff and Erdmann:

Woodruff's *Paramecium aurelia* is evidently a *Paramecium Methuselah* belonging to a non-conjugating line the life history of which is not known in any case.¹¹

It is clear that the cycle emphasized by Maupas, Calkins and others is merely a phantom which has continually receded as each successive investigator has approached the problem with improved culture methods until it has vanished with Woodruff's race of (so far) 4,500 generations. What remains then is the rhythm and in the light of the present study, which demonstrates the underlying cytological phenomena of which it is an outward physiological expression, the whole problem takes on a new aspect. The cell automatically reorganizes itself periodically by a process which, in its main features, simulates conjugation—but without a contribution of nuclear material from another cell. Therefore it is evident (as has been shown by this culture) that the formation of a syncaryon, whose components are derived from two cells, is not necessary for the continued life of the cell—it has an internal regulating phenomenon which is entirely adequate to keep it indefinitely in a perfectly normal condition.¹²

Here we are brought up sharply to face the question which every student of pedigreed infusoria since Maupas has tried to solve. Woodruff and Erdmann conclude from their observations that old age and natural death do not occur in *Paramecium* and that the so-called "cycle" is non-existent. I would draw from their observations exactly the opposite conclusions, viz., that the one apparent exception among pedigreed races, to the rule of depression and natural death in the absence of conjugation or its equivalent, is now removed, and that Woodruff's culture is no more than a long series of cycles.

¹¹ *Ibid.*, p. 429.

¹² Woodruff and Erdmann, p. 489.

We understand by a "cycle," in the sense with which the term was first employed by Calkins, a more or less periodic alternation of high and low vitality as measured by the division rate. The lowering division rate indicates the approach of a period of depression which was interpreted as the equivalent of old age in metazoa, since it indicates a weakening in the chain of vital activities and ends in death unless conjugation or its equivalent is permitted. No one since Maupas, so far as I am aware, has attempted to limit a cycle in terms of definite numbers of generations or definite lengths of time. In 1904 I stated:

The well-marked cycles, therefore, with periods of depression which demanded stimulation of a decided character, were apparently of *six months* duration, while intermediate cycles of less importance were about three months long. . . . During the first three cycles the number of generations was nearly the same (200, 198, and 193, respectively), the last, on the other hand, was much less, the individuals dividing only 126 times.¹³

The period of six months, more or less, or $200 \pm$ generations were not regarded as measures of the cycle, and it was understood at that time that conjugation or its equivalent always inaugurates a new cycle. Woodruff in 1905 introduced the term "rhythm" to designate the lesser periodic fluctuations which I had called "intermediate cycles." Since the entire substance of the much-discussed problem of immortality in infusoria is bound up with this question of the cycle, it is necessary to analyze the so-called rhythms of Woodruff to see how they agree with or differ from the so-called cycles. In *Paramecium* the cycle consists of the history of a bit of protoplasm in an ex-conjugant and its progeny from which conjugation or its equivalent is excluded, until natural death of the entire race ensues. If conjugation or its equivalent occurs the old cycle is abandoned and a new one is started, and there must be as many new cycles as there are times when conjugation or its equivalent takes place. It is imma-

¹³ "Studies on the Life History of the Protozoa," IV. *Jour. Exp. Zool.*, Vol. I, 1904, p. 424.

terial, furthermore, whether such conjugation occurs between individuals of the same race, or between individuals of diverse ancestry, the effect is the same in putting off ultimate weakness and death. With repeated conjugations in such a race the ultimate death may be postponed indefinitely, and this was the argument on which Weismann's revised theory of potential immortality was based.

Now it is exactly the same with Woodruff's rhythms. He finds in his long culture repeated instances of ascending and descending division rates in fairly regular alternate succession. The descending division rate is stopped by an "internal regulatory phenomenon, endomixis."¹⁴ Woodruff and Erdmann, while showing that endomixis is different from conjugation in the absence of a syncaryon, apparently accept it as equivalent to conjugation in connection with vitality of the protoplasm:

Endomixis and conjugation may occur simultaneously in different animals of the same culture, thus strongly suggesting that the same *general* conditions lead to both phenomena—one animal meeting the conditions one way and another by the other, and that *both phenomena fill essentially the same place in the economy of life of Paramecium aurelia*.¹⁵

Again they say:

Endomixis does initiate a new rhythm in the life history of *Paramecium*, *i. e.*, a period of increased metabolic activity and therefore of reproductive activity, and since its fundamental morphological features are almost identical with those preliminary to the formation of the stationary and migratory micronuclei in conjugation, it lends strong support to the view that the dynamic aspect of conjugation is not absent.¹⁶

Throughout the long period of seven years the *Paramecium aurelia* protoplasm without conjugation: "has undergone endomixis frequently, undoubtedly on the average once each month" (*ibid.*, p. 495). Hertwig has already shown, as I do above, that asexual endomixis is parthenogenesis, and if, in connection with the problem of vitality, this is equivalent to conjugation, then we are

¹⁴ *Ibid.*, p. 497.

¹⁵ *Ibid.*, p. 492; the italics at the end are mine.

¹⁶ *Ibid.*, p. 496.

justified in saying that throughout the seven years Woodruff's *Paramecium* has undergone the equivalent of conjugation on the average once each month, and if it is equivalent to conjugation, then his long culture of more than 4500 generations has no bearing on the question of old age and natural death in *Paramecium*.

Nothing in this work of Woodruff and Erdmann seems more clearly and forcibly demonstrated than that the cycle, this "phantom" of many investigators, resolves itself into a demonstrated fact, and that Woodruff's "rhythm" and Calkins's "cycle" are but different names for the same phenomenon. If natural death is a necessary end to justify our use of the term "cycle," we may ask the pertinent question: What happened to those individuals which did not undergo asexual endomixis in Woodruff's long culture? If they died, does not this fact indicate the end of a cycle? If they underwent parthenogenesis, the equivalent of conjugation, does not this fact indicate the beginnings of new cycles? If they continued to live without reorganization, evidence for which has never been given by Woodruff, then there would be some justification for our authors' conclusion. To argue that it is the same race which continues after asexual endomixis is to use the same argument that Weismann used unsuccessfully, viz., that an ex-conjugant is the same old individual since no corpse has been formed and therefore the infusoria are immortal.

The frequent statement made by Woodruff that his long culture sustains the view that old age and need of conjugation are not necessary attributes of living matter are contradicted by these later results. For example, he states in 1913:

Diese Untersuchung hat uns gezeigt, dass, unter günstigen äusseren Umständen, das Protoplasma der zuerst isolierten Zelle mindestens die Potenz hatte, ähnliche Zellen bis zu einer Zahl von 2^{3340} und eine Masse Protoplasma von mehr als 10^{1000} mal der Masse des Erdballes zu erzeugen. Dieses Resultat, glaube ich, bestätigt unzweifelhaft die Annahme, dass das Protoplasma einer einzigen Zelle unter günstigen

äusseren Umständen ohne Hilfe von Konjugation oder einer künstlichen Reizung in stande ist, sich unbegrenzt fortzupflanzen und zeigt ferner in klarer Weise, dass das Altern und das Befruchtungsbedürfnis nicht Grundeigenschaften der lebendigen Substanz sind.¹⁷

I am entirely in sympathy with Hertwig when he says, in connection with this citation:

Nach meiner Ansicht sind die Resultate, zu denen in den unseren Auseinandersetzungen zum Ausgangspunkt dienenden Artikel Woodruff gemeinsam mit Rhoda Erdmann gelangt ist, mit den hier zitierten Sätzen unvereinbar.¹⁸

The discovery of parthenogenesis in the life cycle of *Paramecium aurelia* by Woodruff and Erdmann clears up the obscurity which has involved all theoretical discussions following pedigreed culture work with infusoria, and we now see with much clearer vision the probability, first, that conjugation or its equivalent has primarily the result, as originally interpreted by Bütschli, of offsetting and overcoming the progressive weakening of vitality in infusoria; second, that more or less definite cycles of vigor and depression, ending in natural death unless conjugation or its equivalent supervenes, are characteristic of all pedigreed races of infusoria; third, that physical "immortality" is true of *Paramecium* and other ciliates only in the same sense that it is true of metazoa; fourth and last, that *Paramecium* protoplasm is subject to the same laws of physiological usury that apply to metazoa, and undergoes phenomena which, in metazoa, we call old age, and which, as in metazoa, ends in natural death unless conjugation, or its equivalent parthenogenesis, saves the race.

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¹⁷ Dreitausend und dreihundert Generationen von *Paramecium* u. s. w.,'' *Biol. Centr.*, Vol. 33, No. 1, 1913, p. 35.

¹⁸ "Ueber Parthenogenesis der Infusorien," etc., *Biol. Centr.*, Vol. 34, No. 9, 1914, p. 577.